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13. ABSTRACT (Maximum 200 Words)

Intraductal "foam cells" are the most commonly encountered cells in spontaneous nipple discharge, nipple aspirate fluid and ductal lavage yet their origin and significance remain a mystery. They frequently surround DCIS and other intraductal proliferations but their presence has been regarded as a nuisance since they often hide the diagnostically more important epithelial cells. Our previous immunocytochemical studies with macrophage (CD68, lysozyme), epithelial (cytokeratin, estrogen receptor) and myoepithelial (smooth muscle actin, CALLA, maspin) markers have indicated that foam cells are of macrophage lineage and terminally differentiated (negative Ki-67 and PCNA). Their origin has been presumed to be of ductal lining epithelium. However this has not been proven. The origin and significance of mammary intraductal foam cells remain an important and unanswered question warranting study. Specifically our "Concept" proposal tests the hypothesis that these intraductal macrophages take origin from bone marrow–derived hematopoietic precursors. Bone marrow from wild type male C57 black mice and green fluorescent protein (GFP)-transgenic male C57 black mice (Jackson Laboratories) will be harvested by femoral flushing. In the former group, the bone marrow will be labelled *ex vivo* by retroviral transfection of GFP using the latest generation of modified retroviral vectors designed to maximize gene expression in hematopoietic stem cells. In both groups, the harvested marrow will be tail-vein injected into lethally irradiated female C57 mice. Mice exhibiting successful bone marrow engraftment of at least 50% donor marrow will be identified and made pseudopregnant with injections of progesterone and their mammary fat pads will be excised and examined. The presence of GFP intraductal macrophages will be searched for.

14. SUBJECT TERMS
Intraductal foam cells, macrophages, bone marrow, precursor cells, GFP-labeled stem cells

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INTRODUCTION

Background: Intraductal "foam cells" are the most commonly encountered cells in spontaneous nipple discharge, nipple aspirate fluid and ductal lavage yet their origin and significance remain a mystery (1). These cells increase in human and murine pregnancy and other conditions of ductal ectasia and / or obstruction. They frequently surround DCIS and other intraductal proliferations but their presence has been regarded, more or less, as a nuisance since they often hide the diagnostically more important epithelial cells (2). Our previous immunocytochemical studies with macrophage (CD68, lysozyme), epithelial (cytokeratin, estrogen receptor) and myoepithelial (smooth muscle actin, CALLA, maspin) markers have indicated that foam cells are of macrophage lineage and terminally differentiated (negative Ki-67 and PCNA). Studies of others have confirmed these observations (3). We have also observed in humans other variants of these intraductal macrophages which exhibit not foam vacuoles of milk / milk products but phagocytosed endogenous intraductal debri including erythrocytes / hemosiderin and exogenous materials including barium and gastrograffin introduced by ductal lavage (2).

BODY (STATEMENT OF WORK)

Rationale/Purpose: Because these macrophages are observed only intraductally and because their appearance resembles lactating and vacuolated epithelial cells, their origin has been presumed to be of ductal lining epithelium (3). However this has not been proven. The origin and significance of mammary intraductal foam cells remain an important and unanswered question warranting study.

Objectives: Specifically our "Concept" proposal tests the hypothesis that these intraductal macrophages take origin from bone marrow–derived hematopoietic precursors. We shall limit our studies in this proposal to murine bone marrow transplant models but if these studies yield promising results, we will examine the hypothesis in humans in a subsequent grant proposal of a different type.

Methods: Bone marrow from wild type male C57 black mice and green fluorescent protein (GFP)-transgenic male C57 black mice (Jackson Laboratories) will be harvested by femoral flushing. In the former group, the bone marrow will be labelled *ex vivo* by retroviral transfection of GFP using the latest generation of modified retroviral vectors designed to maximize gene expression in hematopoietic stem cells (4,5). In the transgenic group *ex vivo* labelling will not be necessary. In both groups, the harvested marrow will be tail-vein injected into lethally irradiated female C57 mice, a technique which will destroy most of the recipient marrow. Mice exhibiting successful bone marrow engraftment of at least 50% donor marrow will be identified and made pseudopregnant with injections of progesterone and their mammary fat pads will be excised and examined. The presence of GFP-containing intraductal macrophages will be searched for and if found, their donor bone marrow origin will be further confirmed by investigating whether they contain a second donor marker (the Y male chromosome) by fluorescence in situ hybridization (FISH) studies. As additional controls for the significance of our findings, the presence of free GFP within ductal fluid will be investigated. Furthermore tail vein injections of GFP-labelled murine lymphocytes and embryonal fibroblasts into wild type female C57 mice will be performed to see whether these latter maneuvers also produce GFP-containing intraductal macrophages. If both these sets of control experiments prove negative, that will indicate that the GFP observed within intraductal macrophages is not the result of phagocytosis of free GFP but rather the result of an origin from GFP-labelled bone marrow macrophage precursor cells. If the reporter gene

experiments of bone marrow prove that marrow cells become intraductal macrophages, we will replace the *ex vivo* retroviral transfections of reporter genes like GFP with genes of interest that have known

effects on breast epithelium (proliferation *v* apoptosis, eg TGF-alpha *v* TGF-beta). We will observe the biological effects of these manipulations on the murinebreast with the appropriate immunocytochemical assays (Ki-67 *v* TUNEL). If these latter experiments prove successful, future diagnostic and / or therapeutic strategies in humans could be based on the omnipresence of intraductal macrophages in the breast and their bone marrow origin. *Ex vivo* labelled bone marrow stem cells, for example, could be made to localize as intraductal macrophages and deliver their therapeutic genes of interest to DCIS, other precancerous lesions or high risk ductal epithelium.

KEY RESEARCH ACCOMPLISHMENTS

The study was temporarily suspended during the past year because of unresolved Animal Research Committee issues. As a result no key research accomplishments occurred. These animal issues were recently completely resolved and the study can now progress uninterrupted.

A no cost extension was sought and granted. It is my expectation that during this no cost extension period that significant research accomplishments will occur.

REPORTABLE OUTCOMES

None

CONCLUSIONS

Pending

PERSONNEL

Sanford H. Barsky, M.D., Principal Investigator
Jian Yu Rao, M.D., Director, Animal Compliance
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